

# COMSTAT 2

## A biofilm quantification program

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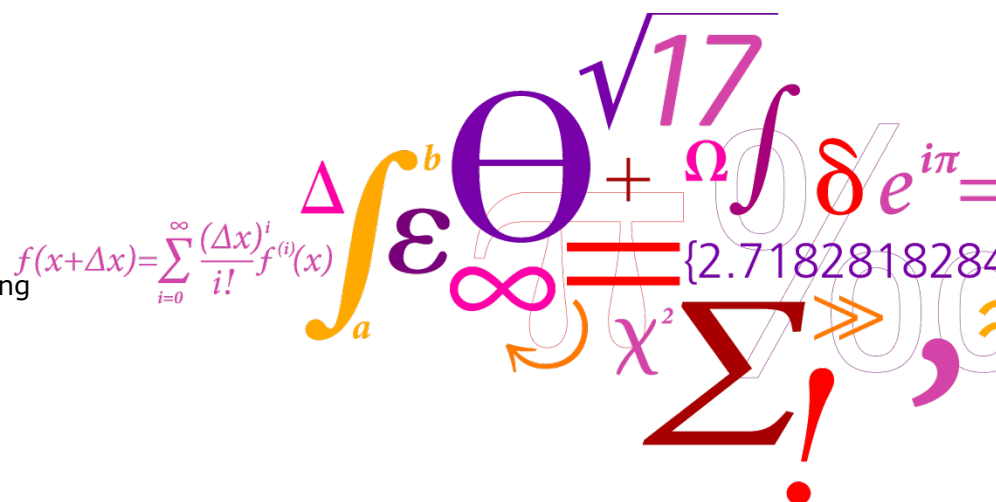
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**DTU Systems Biology**

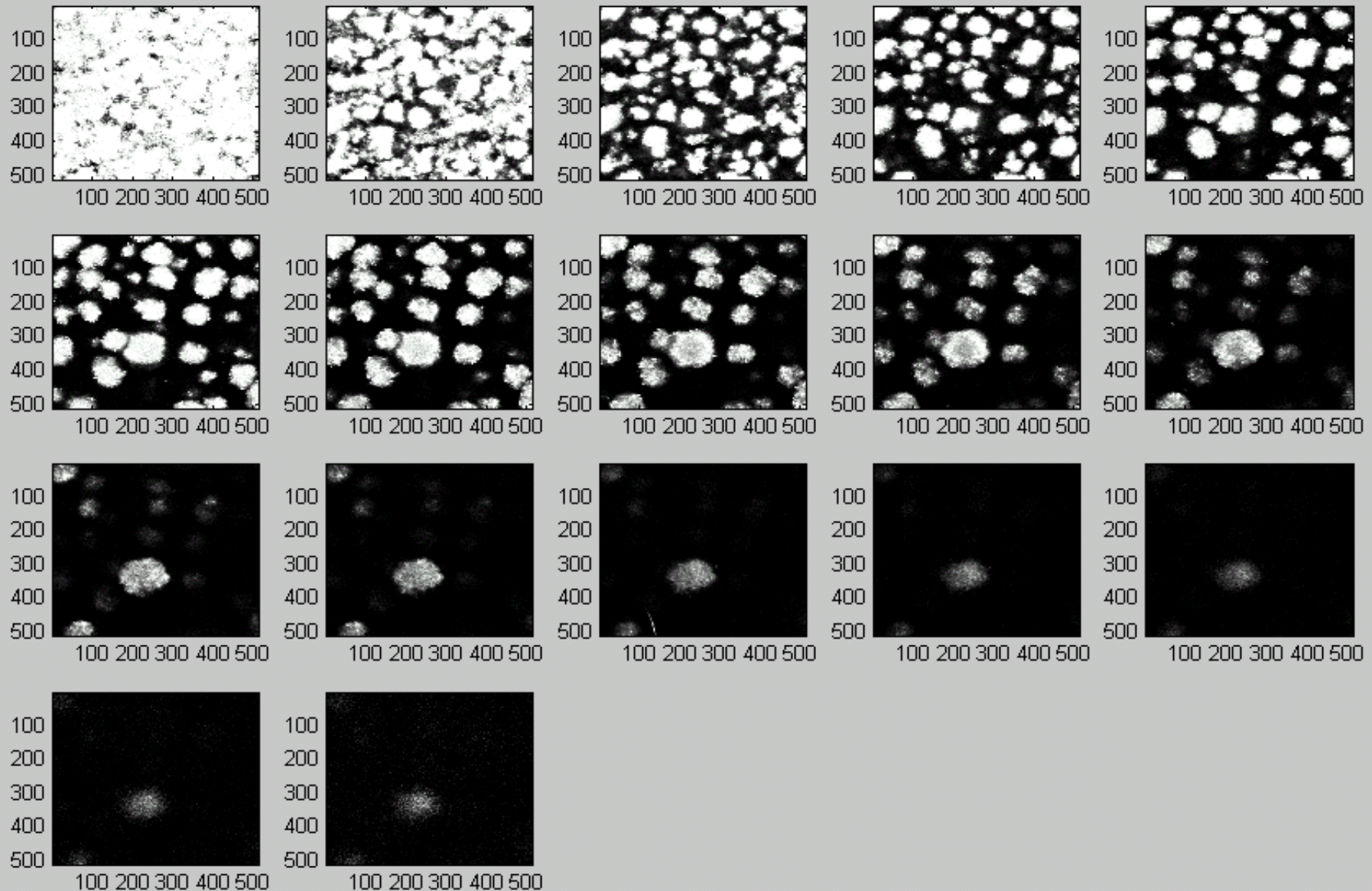
Department of Systems Biology



We want to be able to perform unbiased and reproducible biofilm experiments

# Biofilm data from CLSM

su040609.info - original



# Biofilm

## Descriptive parameters

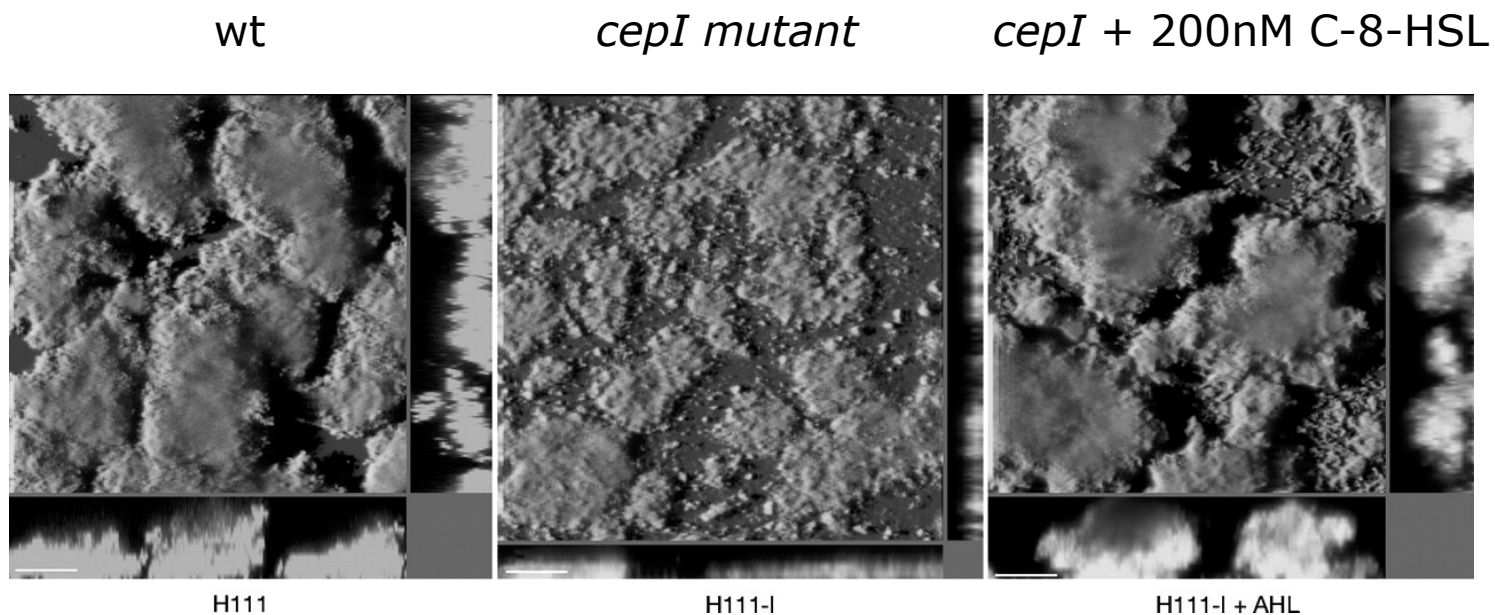
Which species are present?

What is the structure (the 3-dimensional distribution) of the species in the biofilm?

What is the individual bacterium doing?

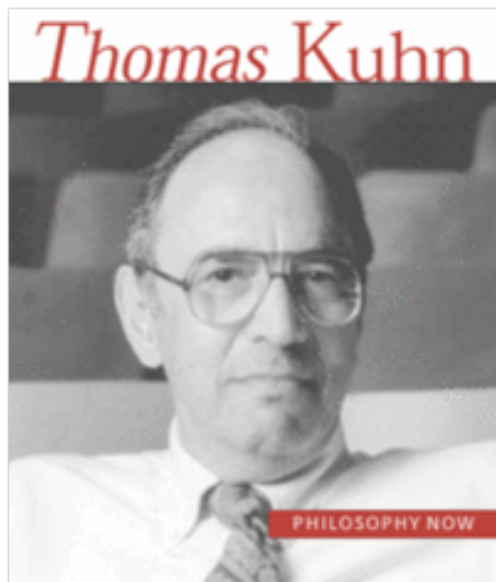
Do they communicate?

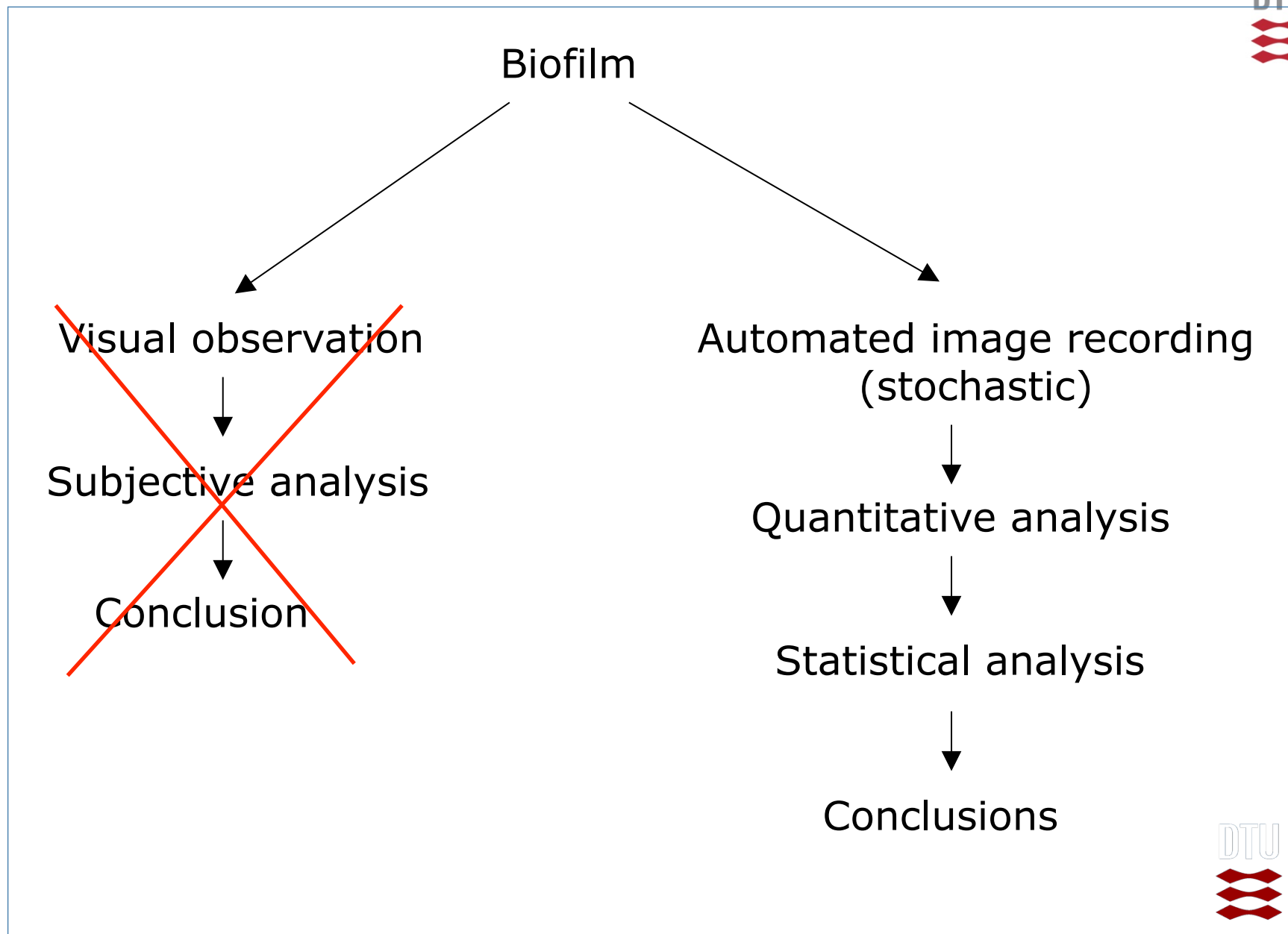
# Quantifying biofilm structures



What a man sees depend both of what he is looking at and what his previous visual-conceptual experience has taught him to see.

*Thomas Samuel Kuhn, 1970*



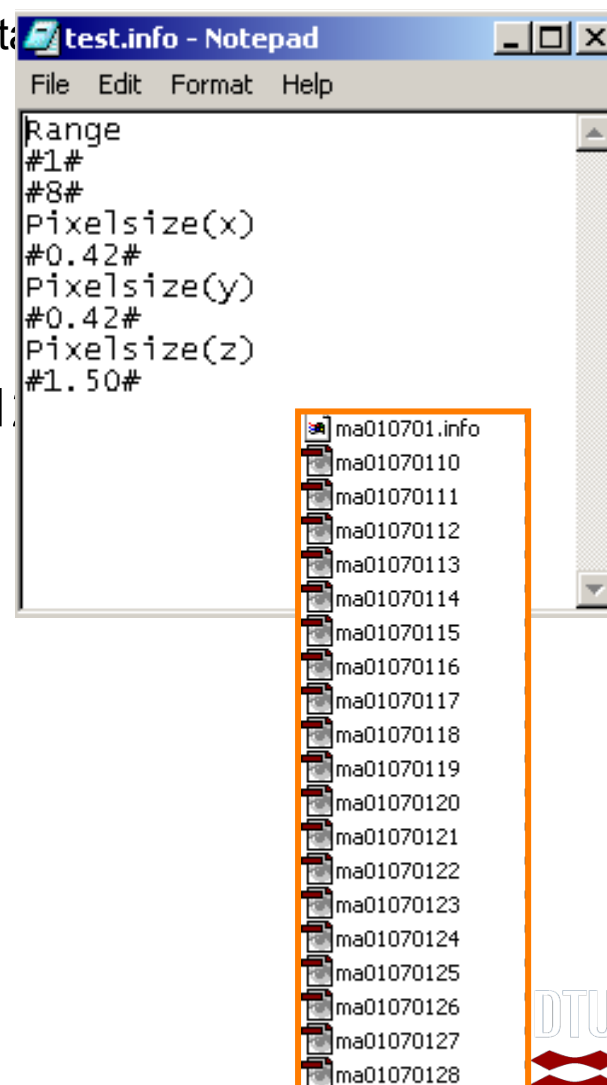


# COMSTAT 1

– processing of image data

- File format (TIFF)
- info-file (pixelsize, number of sections) – legacy Leica format
- Filename-syntax (xxx10.tiff, xxx11.tiff, xxx12.tiff, ...)
- Thresholding ('Look' function)
  - noise filtering
  - binary conversion
  - connected volume filtering (?)

→ Run COMSTAT in MATLAB





# COMSTAT 1

## Quantifying biofilm structure

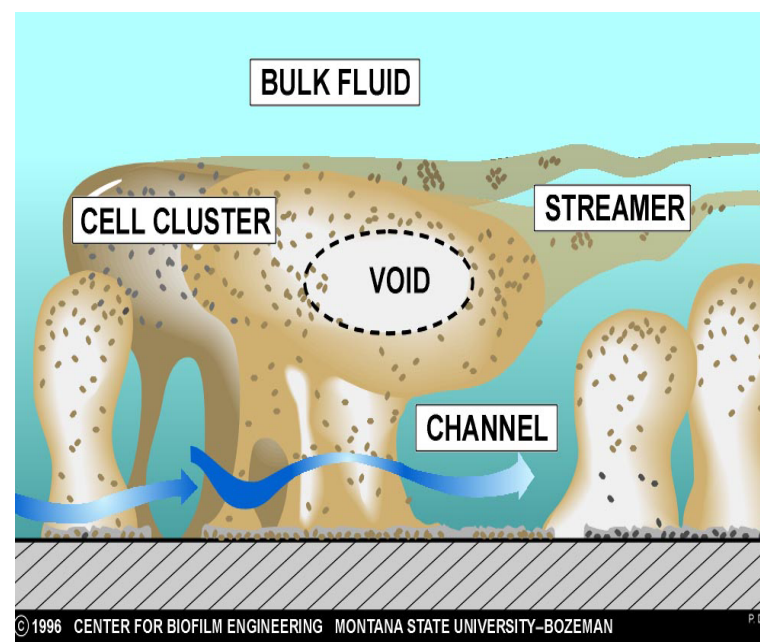
Quantitative measurement vs. subjective observation

The first 3D biofilm quantification program

Written as a MATLAB® script

COMSTAT 1 can quantify biofilm images captured using a confocal microscope:

- Biofilm thickness
- Biovolume (“Biomass”)
- Roughness
- Surface to Volume ratio
- Substratum coverage
- Number of micro colonies
- Micro colony size
- Distribution of diffusion distances
- Fractal dimension (not really)



# COMSTAT

– parameters calculated

- **Bio-volume** ( $\mu\text{m}^3/\mu\text{m}^2$ )

Biomass volume divided by substratum area - provides an estimate of the biomass in the biofilm.

- **Area occupied by bacteria in each layer** ( $\mu\text{m}^2/\mu\text{m}^2$ , dimensionless)

Substratum coverage is the area-coverage at the base of the biofilm.

- **Thickness distribution and average thickness**

(The thickness measure ignores the presence of pores or voids in the biofilm)

# COMSTAT

– parameters calculated

- **Identification and area distribution of micro-colonies at the substratum**

- A minimum micro-colony size must be specified.
- The function calculates the total number of micro-colonies, the area size of each micro-colony ( $\mu\text{m}^2$ ) and the average micro-colony area ( $\mu\text{m}^2$ ).

- **Volumes of microcolonies identified at the substratum**

- This function calculates the volume ( $\mu\text{m}^3$ ) of each of the micro-colonies identified above and the average micro-colony volume ( $\mu\text{m}^3$ ).

# COMSTAT

– parameters calculated

- **Fractal dimension**

- **Roughness coefficient** (variation in thickness)

calculated from the thickness distribution of the biofilm

$$R_a^* = \frac{1}{N} \sum_{i=1}^N \frac{|L_{fi} - \bar{L}_f|}{\bar{L}_f}$$

- $L_{fi}$  is the  $i$ 'th individual thickness measurement
- over-lined  $\bar{L}_f$  is the average thickness
- $N$  is the number of thickness measurements.

→ Biofilm roughness provides a measure of how much the thickness of the biofilm varies, and is an indicator of biofilm heterogeneity.

# COMSTAT

– parameters calculated

- **Distribution of diffusion distances, average and maximum diffusion distance**

The diffusion distance for a voxel containing bio-mass is the shortest distance from that voxel to a voxel not containing bio-mass (void)

Average and maximum diffusion distances have been suggested as measures of the distances, over which nutrients and other substrate components have to diffuse from the voids to the bacteria within micro-colonies

- **Surface to volume ratio** (surface area/bio-volume,  $\mu\text{m}^2/\mu\text{m}^3$ )

-reflects what fraction of the biofilm is in fact exposed to the nutrient flow  
(How does the biofilm adapt to the environment? Does a low nutrient environments lead to an increased surface to volume ratio to optimize access to the limited supply of nutrients?)

# Example

Three independent experiment rounds

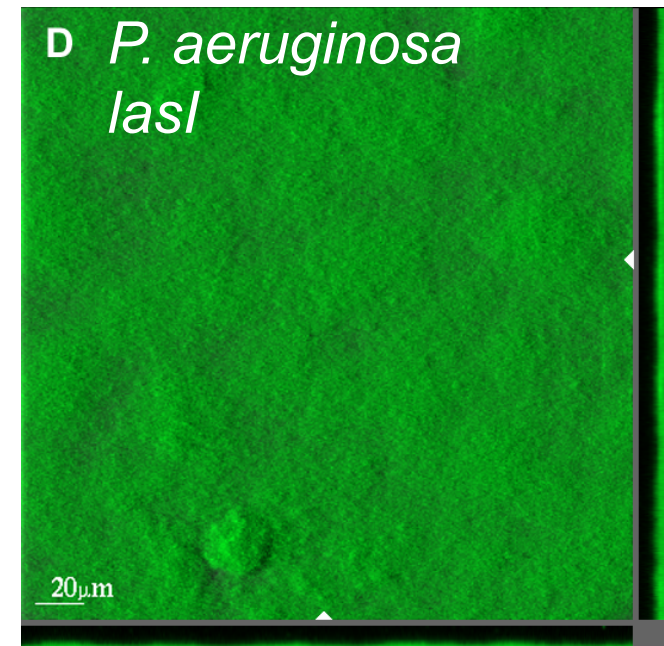
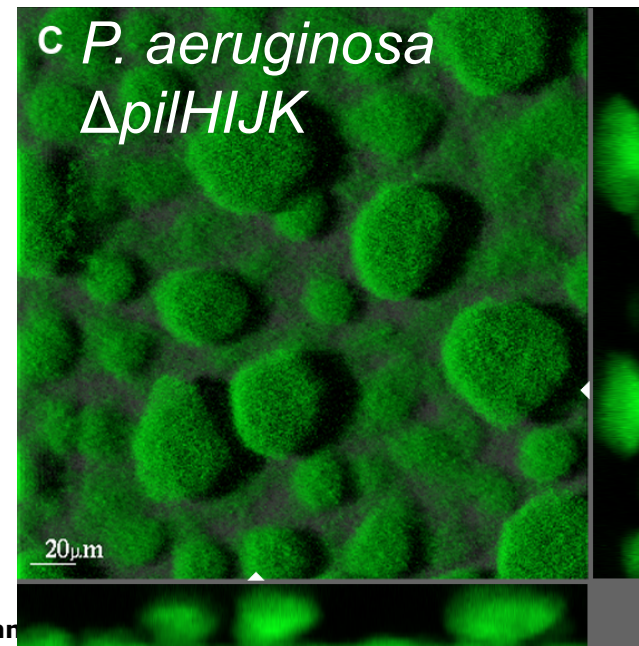
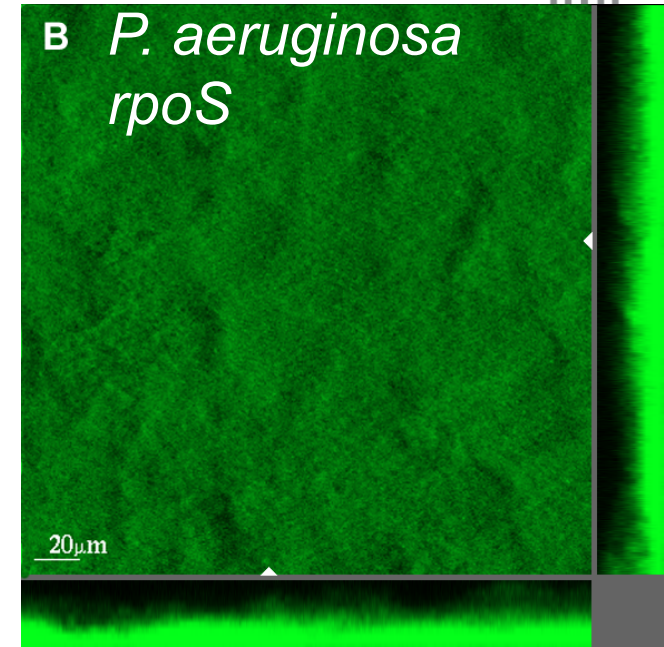
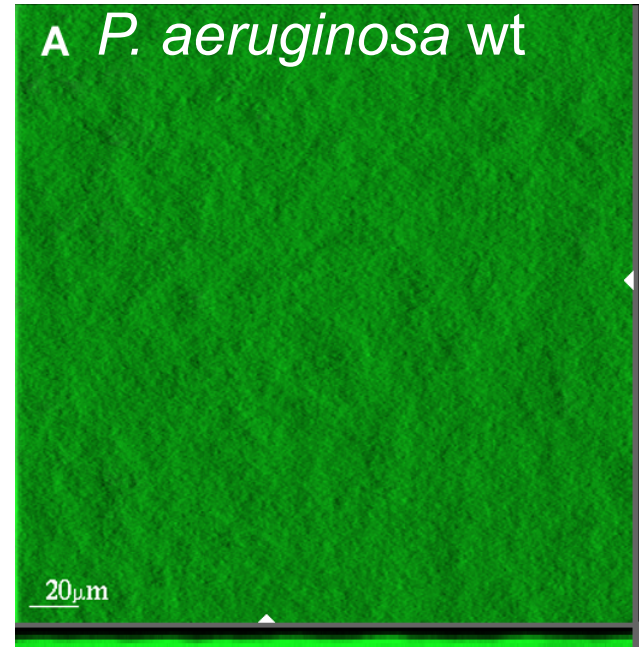
Each experiment:

- Four strains of *P. aeruginosa*, each in two channels equals 8 channels
- Five time points: 55h, 98h, 146h, 242h, 314h
- Nine image stacks in each channel at each time point

Total: 1080 image stacks

Images are acquired at random spots at a distance of 5-10 mm from the inlet to the flow-channels.

Flow chamber biofilms  
of *P. aeruginosa*  
146 hours after  
inoculation

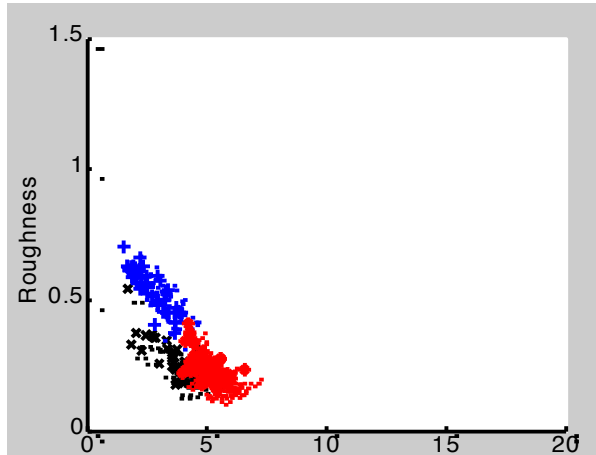




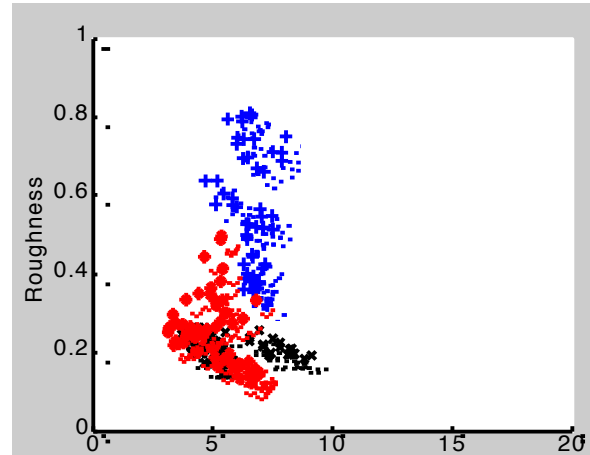
# COMSTAT analysis of *P. aeruginosa* strains



55 hours

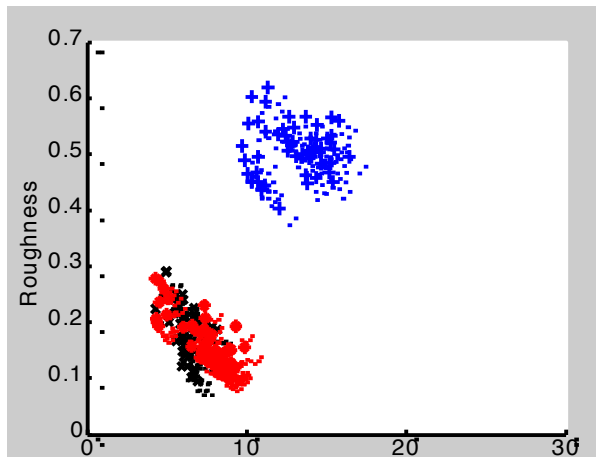


98 hours

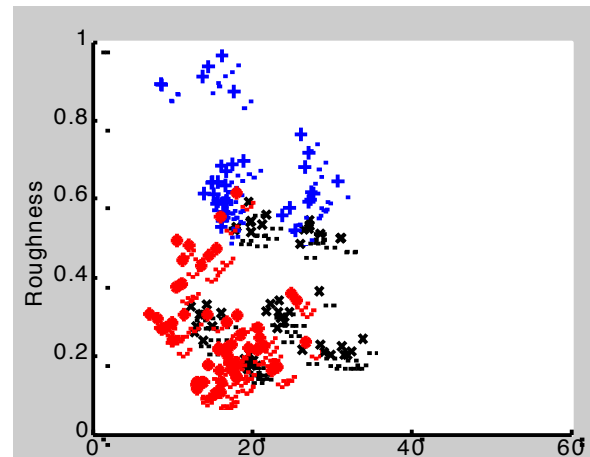


× *P. aeruginosa* PAO1  
○ *P. aeruginosa* *rpoS*  
+ *P. aeruginosa*  $\Delta pilHIJK$   
\* *P. aeruginosa* *lasI*

146 hours



314 hours





# Statistical analysis of biofilm structure

## Variance model

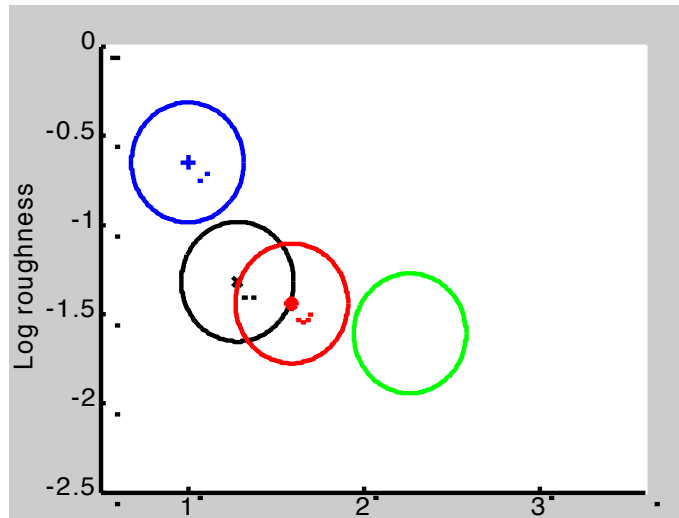
$$Y_{ijkv} = \mu + b_i + R_j + BR_{ij} + C(BR)_{k(ij)} + Z_{v(ijk)}$$

- $Y_{ijkv}$  : Observed value for bacterial strain  $i$ , experiment round  $j$ , channel number  $k$ , and image stack  $v$ .
- $\mu$  : Overall mean value of the experiment
- $b$  : Additional effect of bacterial strain  $i$  (strain  $i = 1, 2, \dots$ )
- $R_j$  : Random effect of experiment round  $j$  (round  $j = 1, 2, \dots$ )
- $BR_{ij}$  : Random effect of a possible interaction between bacterial strain  $i$  and round  $j$
- $C(BR)_{k(ij)}$  : Random effect of channel  $k$  (channel  $k = 1, 2, \dots$ )
- $Z_{v(ijk)}$  : Residual error of observation (strain  $i$ , round  $j$ , channel  $k$ )

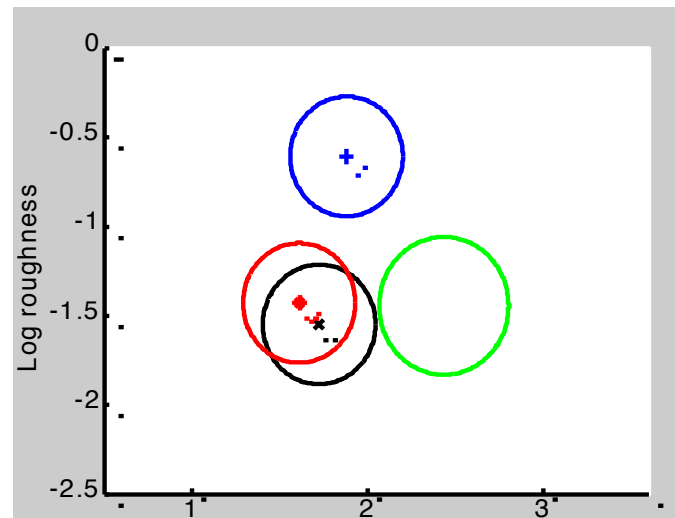
# Comparison of *P. aeruginosa* biofilm architecture



55 hours

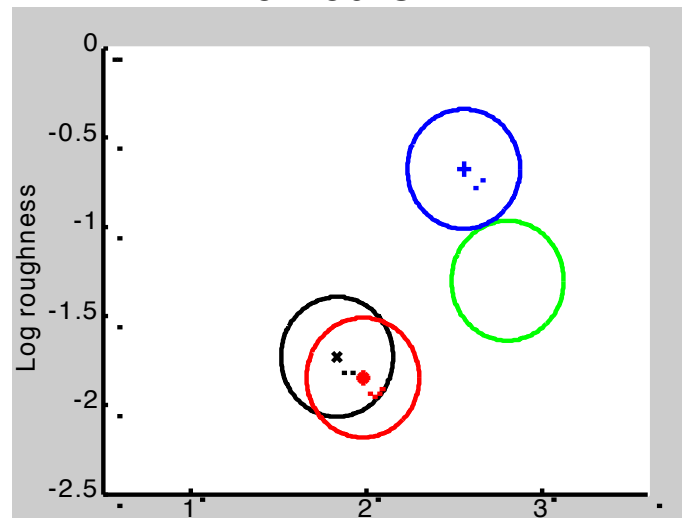


98 hours

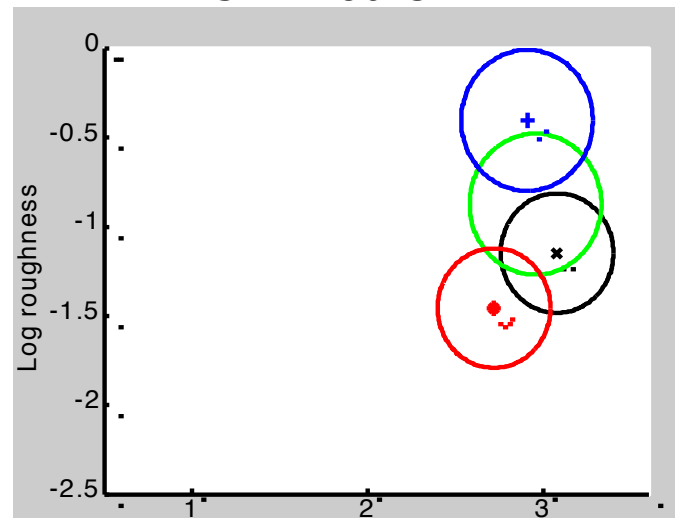


× *P. aeruginosa* PAO1  
 o *P. aeruginosa* *rpoS*  
 + *P. aeruginosa*  $\Delta pilHIJK$   
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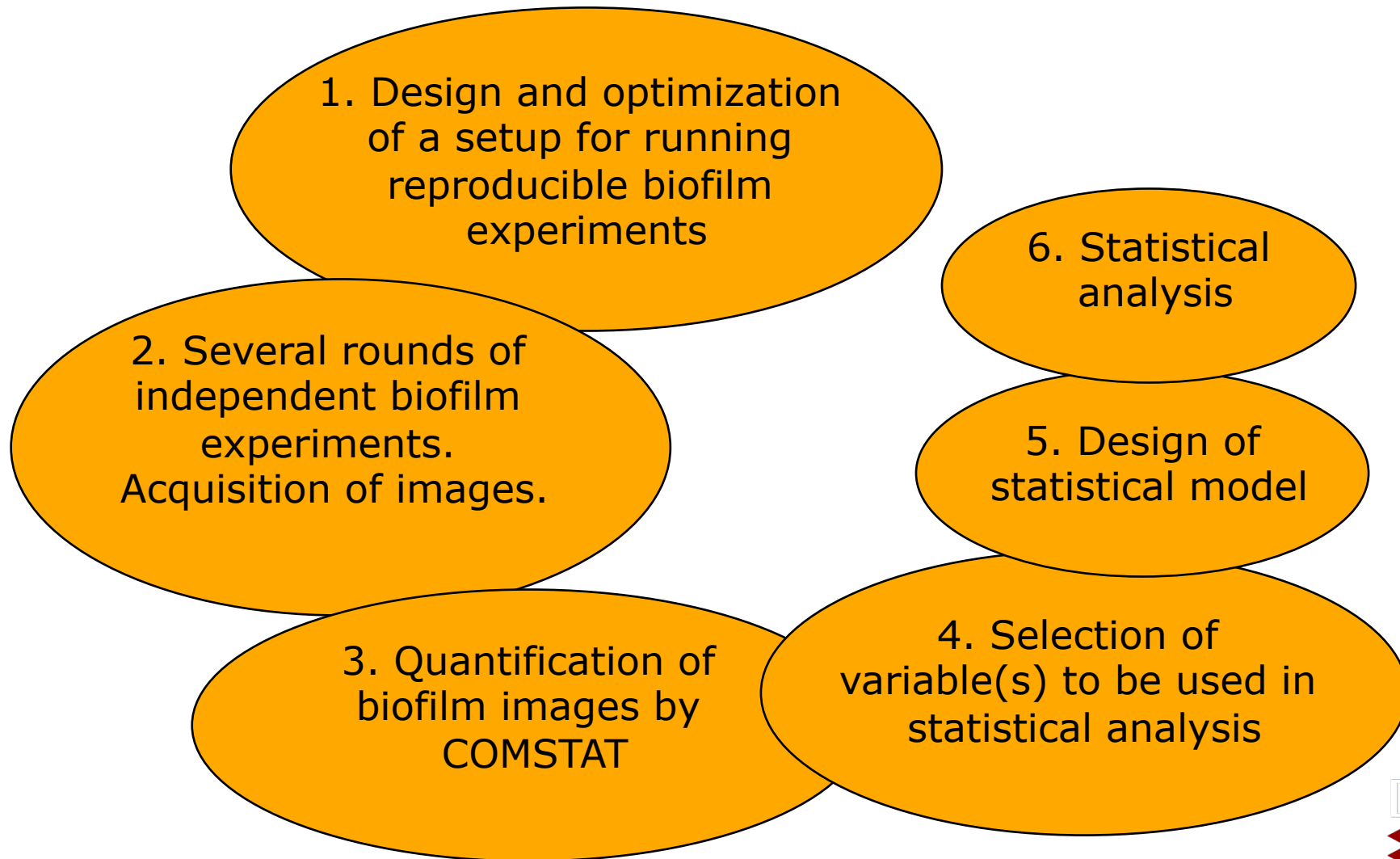
146 hours



314 hours



# Quantification and statistical analysis of biofilm structures



# LIVE DEMO